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Case review

Deletion of amelogenin Y-locus in forensics: Literature revision and description of a novel method for sex confirmation

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ABSTRACT

Today, the molecular technique routinely for sex determination in forensics is based the detection of length variations in the X–Y homologous amelogenin gene (AMELX and AMELY).

In humans, the amelogenin gene is a single-copy gene located on Xp22.1–Xp22.3 and Yp11.2; the simultaneous detection of the X and Y alleles using polymerase chain reaction (PCR) can lead to gender determination.

Several studies have shown that normal males may be typed as females with this test: AMELY deletions may result in no product of amplification and normal males being typed as female as a result of the test (negative male).

Considering the consequences of the result obtained using only the amelogenin marker, and the related potential difficulties in interpreting the results, the gender misinterpretation may be troublesome in clinical practice and in forensic casework.

In this article, beginning with a review of the incidence of gender-testing failures among different populations, and with the different strategies proposed in the literature in case of doubt regarding the presence of deleted AMEL in the DNA profile, we propose a method for the identification of samples with deleted AMEL that can be applied, as an additional assay, in case of doubt regarding PCR results of sex determination.

1. Introduction

The human amelogenin gene, sequenced by Nakahori et al. in 1991,[1] which is highly conserved in primates,[2] is located on both the X and Y chromosomes: the AMELX gene has a size of 2872 bp and is located on the p22 region of the X chromosome, while the AMELY gene has a size of 3272 bp and is located on the 11p12.2 region of the Y chromosome.

Amelogenin is a gene that codes for a matrix protein forming tooth enamel, in which it represents 90% of the organic content.[3]

Polymerase chain reaction (PCR) products generated from AMELX and AMELY chromosomes can be discriminated from one another using primers flanking a 6-base-pair (bp) deletion in the first intron of the sex chromosomes. Sequence differences between the X and Y homologues of the amelogenin gene have been used to differentiate, in molecular analysis, males from females with ambiguous phenotype, or to establish the gender in biological-material analysis for different purposes. Since both X- and Y-specific fragments can be amplified in a single reaction, amplification of the amelogenin gene offers the advantage of having an internal positive control represented by the X-chromosome homologous fragment which should always be present. The two most commonly used amelogenin primer sets span a 6-bp deletion on the X chromosome and generate fragments of 106/112 bp or 212/218 bp for X/Y products, respectively.[4,5]

Several companies manufacture amelogenin primer sets for sex identification, as well as multiplex short tandem repeat (STR) kits, which contain primers specific for the amelogenin gene, which allows for individual as well as gender identification.

In forensics, the use of multiplex-PCR systems with additional amelogenin detections leads to sex determination of organic traces. It is useful to distinguish between the victim and the perpetrator's evidence, in case of sex determination of remains in mass disasters

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or missing persons' investigations and particularly in sexual assault cases. This method of detection can also help to resolve sample mix-ups and studies of old bones which lack clear morphological diagnostic features, such as in the case of fragmentary or juvenile skeletal remains.

A particular situation which must be highlighted, involving both forensic and clinical aspects, is that which occurs after successful allogenic bone marrow transplantation in sex-mismatched donor–recipient pairs. The amelogenin-based sex determined in the blood samples (haematopoietic cell lines) of the recipient is the same as that of the donor, but it is different from the sex determined in the DNA obtained from the recipient's structural cells.[6]

1.1. Pitfalls in gender determination: AMELY dropout

Several studies in the literature to date have reported mutations in the AMELY homologue gene which can lead to the typing of males as females, with noteworthy consequences if used in criminal investigations (forensic/rape cases) or in the identification of human remains after mass disasters.

For the first time in scientific literature Santos et al. reported that the amelogenin-based sex test incorrectly types some males as females because they lack the AMELY as a result of a deletion. They considered 350 individuals coming from different countries and reported two cases of amelogenin-based sex-test failure: they both came from Sri Lanka (0.6% frequency of sex-test failures due to deletion polymorphisms in the sample). The same authors observed a deletion frequency of 8% within their Sri Lankan population sample ($n = 24$) suggesting that in some populations the frequency of failure might be higher; they proposed as the mutation mechanism a recombination between the test is-specific Y-encoded protein A (TSPYA) and TSPYB loci.[7]

Other cases of AMELY mutations have been reported[8,9] and some authors have argued that the AMELY null result might be consequent to a point mutation within the primer binding-site region, rather than a deletion of the AMELY sequence.[9,10]

Thangaraj et al. investigated a total of 270 Indian male samples finding that five individuals showed deletion polymorphisms of about 1 Mb encompassing the AMELY locus on Yp, defined through Southern hybridisation, with an observed amplification failure frequency of 1.85%.[11] Steinlechner and collaborators reported that in a population sample of 29,432 phenotypic males stored in the Austrian National DNA database, six individuals lacked the AMELY PCR amplification, resulting in wrong gender determination with an observed frequency of amelogenin-based sex-test failure of 0.018%. In this study the sex could be correctly determined by typing Y-STR loci in five cases and in one case by typing sex-determination region Y (SRY).[12]

Chang et al. in 2003 described a 3.6% failure rate of the amelogenin-based sex test in an Indian population group from Malaysia (population sample of 338 males, 113 Malays, 113 Chinese and 112 Indians) and they found the dropout of both AMELY and MSY1 minisatellite loci, showing that the deletion spanned a length in the order of probably several kb.[13] A bigger Malaysian population group ($n = 980$, 334 Malays, 331 Chinese and 315 Indians) was studied in 2007, showing that both the Indians and Malays exhibited amelogenin-null frequency of 3.2% and 0.6%, respectively. The two studied groups identified a similar deletion region on the Yp11.2 band of at least 1.13 Mb, encompassing the AMELY gene, MSY1 minisatellite and the DYS458 locus. The haplogroup results for the Indian null results indicated an ancestral J2e lineage.[14]

As failure in gender determination was found to be particularly high in males originating from India, Kashyap et al. analysed the amelogenin typing results of 4257 males originating from different regions of India and included in the DNA databasing project. Among these individuals, 10 confirmed males showed a dropout of the 112-bp AMELY gene and the overall failure rate was 0.23%. They were tested with alternate primer pairs encompassing the previously amplified region and they failed amplification. Additional analysis with male-specific SRY loci, with four Y-specific STR polymorphisms and Y-single nucleotide polymorphisms (SNPs) were performed and the authors inferred that the deletion spanned a region downstream of the reverse primer-binding site of commercially available amelogenin primer sets.[15]

In 2004, the Israel Defence Force reported the failure of an amelogenin-based sex test on a phenotypically normal male soldier.[16]

Lattanzi et al. described an interstitial deletion of the Y short arm encompassing the AMELY locus in two cases of unrelated individuals: one case was identified during the screening for Y microdeletions performed on a sample of 493 infertile males while the second one was found among 13,000 amniotic liquid samples from male foetus pregnancies tested by quantitative fluorescence-polymerase chain reaction (QF-PCR) and cytogenetic analysis for prenatal diagnosis. In order to estimate the extent of the deletion on Yp, the researchers performed a pulsed-field gel electrophoresis (PFGE), followed by fluorescence *in situ* hybridisation (FISH) and sequence tagged site (STS) marker analysis and the deletion size was about 2.5 Mb. An intact SRY locus was present in both individuals and, considering that the two carriers of the deletion had different Y-STR haplotypes, it was supposed that the mutation has occurred independently or that there has been an old single mutational event.[17]

In Australia the frequency of AMELY amplification dropout reported by Mitchell et al. was low (0.02%) in a population sample of 109,000 males. The samples of five AMELY-null males (two of Indian origin, one Italian and two South-Asian) were typed for eight STSs located on Yp using PCR and gel electrophoresis and also for 11 Y-specific STRs to study haplotypes for phylogenetic analysis. In the five samples two different-sized deletions were identified (ranging between 304–731 and 712–1001 kb).[18]

In a sample population of 77 males from Kathmandu, Nepal, five null AMELY individuals were found (6.49%). In this case the deletion size was estimated to be of about 2.3 Mb, through a battery of male-specific SNPs, STRs, STSs and minisatellites; furthermore, considering the Y-haplogroup analysis performed, all five individuals were shown to belong to the same lineage (J2b2-M241).[19] Literature data suggest a concentration of AMELY-null individuals in the Indian subcontinent, possibly as a result of common ancestry.

Jobling et al. analysed a total of 45 AMELY-null males from different origins (among these, 32 have already been reported in the literature by other researchers) and studied a combination of STS deletion mapping, binary-marker and Y-haplotyping and TSPY copy number estimation to understand the structural basis of the deletions involved. The authors reported four different deletion classes, ranging from 2.5 Mb to 4.0 Mb, due to a total of 10 independent events.[20]

Two cases of AMELY deletion have been reported in the north-east Italian population: in the first case an alleged father–son pair was erroneously typed as females. In the second case the AMELY deletion was found in a sample of amniotic fluid collected during prenatal diagnosis of a male foetus pregnancy. The authors suggested a deletion range of 3.35–3.87 Mb in the first case and 1.51–2.58 Mb in the second case, respectively. Furthermore, the two cases showed different haplotypes and different deletion breakpoints.[21]

In a Japanese casework involving two dead brothers and two of their male relatives, all four males were lacking the amelogenin Y homologue. Investigation using Y-specific markers showed a deletion of about 2.56 Mb in the Yp11.2 region.[22] Thereafter, the

same research group found another case of a Japanese AMELY-null male and they performed, in both cases, the high-resolution STS mapping and the Y-haplogroup analysis. They argued a deletion mechanism of non-homologous end-joining, and sustained that the two deletion events had occurred independently.[23] Another case of an amelogenin-null male was reported in Japan and the sizes of the identified deletions were approximately of 2.51 Mb, 25 kb and 834 b and the Y-STR haplotype was different from those previously reported in the literature.[24]

Considering the different deletion distribution among the populations reported in scientific literature, we can conclude that the observed frequency of the amelogenin sex-testing failures shows a population-specific difference.

1.2. Pitfalls in gender determination: AMELX dropout

While the AMELY deletion may lead to incorrect determination of sex, the AMELX deletion is less troublesome in amelogenin-based sex tests: in fact, in the case of a true female subject the AMELX deletion in one X chromosome remains undetected, while the PCR dropout of both X-homologous amelogenin loci in a true female subject clearly suggests that there have been some problems in gender determination by using the amelogenin gene, because there will be no results in the electrophoresis pattern. In the case of a true male subject there will be the only AMELY fragment amplification, suggesting the failure of AMELX amplification.

The PCR drop-out of the X-homologue of the amelogenin gene has been reported rarely in the literature, often as a consequence of point mutations in PCR primer binding sites.[25,26] Caratti et al., testing over 43,000 individuals through routine QF-PCR, observed a single Caucasian male originating from northeast Italy lacking amplification AMELX with normal genotypes at pseudoautosomal and X-chromosomal STR loci.[27] In this case the cause of the AMELX drop-out was identified in a point mutation in the annealing region of primers included in the amplification kit, rather than a deletion. The mutation described by Caratti et al.[27] was identical to that reported by Shadrach et al.[25] in a Caucasian (a population sample of 327 individuals of unspecified ethnic origin from the United States of America) and by Maciejewska and Pawlowski in a Caucasian male (population sample of 5534 Polish males).[28]

1.3. Strategies to avoid misinterpretation in case of null-AMELY (males)

In order to solve the problem encountered with null-AMELY males, in forensics, different approaches have been settled on for gender determination/confirmation.

Haas-Rocholz and Weiler investigated the size of flanking sequences of homologous regions of the AMELX and the AMELY gene for possible primers producing PCR fragments smaller than those described by Sullivan,[4] and they suggested primers AMELU1 and AMELD1, resulting in an 80-bp X-chromosome-specific and an 83-bp Y-chromosome-specific fragment.[29] However, for the reasons mentioned above, even with these shorter PCR products in the amelogenin-based sex test the possibility of incorrect gender determination remains.

Some studies have investigated the use of genetic markers lying in the SRY of the Y chromosome as additional singleplex PCR first and then in combination with AMEL primers, but these procedures were more lengthy, more time consuming and used up a greater sample amount.[11,20,30]

Nevertheless this method, if used as a singleplex of SRY, cannot accurately indicate a female genotype because the amplicon may be absent due to degraded DNA in the examined specimen.

On the other hand, if SRY is co-amplified with AMEL, it helps detection of AMELY-null samples, so some different authors have proposed in the literature different methods for associating a Y-specific marker to AMEL.

SRY is thought to direct the sex-determination pathway towards male development. It has been demonstrated[31] and validated[32] that the SRY gene assay for gender determination can be incorporated into other STR analysis with commercial kits containing also AMEL. The advantage of this assay is that the PCR product of this novel marker residing in the SRY gene is 96 bp long, thus providing feasible gender determination when typing degraded forensic samples. Furthermore, the SRY amplification product does not migrate with any of the AMEL or STR alleles in the multiplex STR kits because of its short length; hence, it can be included in multiplex kits.

A completely different approach is the one proposed by Andréasson and Allen,[33] in which the sex-determination test is performed with a real-time PCR assay, based on melting-curve analysis, while an externally standardised kinetic analysis allows quantification of the nuclear DNA copy number in the sample.

Morikawa et al. have developed a method for sex determination using a multiplex amplification of SRY, STS (steroid sulphatase) and amelogenin gene regions and their homologous sequences. For this method a detection limit of 63 pg of genomic DNA was demonstrated, and the male DNA component could be detected from mixed samples with a male:female ratio as low as 1:10.[34]

Furthermore, the pentanucleotide microsatellite DXYS156 maps to the pseudoautosomal region of both sex chromosomes and can be used for sex determination.[35] DXYS156 is a multi-allelic STR with geographic-specific allelic distribution, helping not only the determination of sex but also of the probable geographical origin of an individual. The Y-specific alleles can be distinguished from the X-specific alleles because of an adenine insertion in the repeat units of the STR.[36]

In this article we propose a method for the identification of sample with deleted AMELY based on a small polyacrylamide-gel electrophoresis of a duplex PCR product which has never been used for this purpose so far. These amplicons correspond to the AMEL gene and to a 197-bp-long PCR product of the SRY gene.

This article shows that the method can be applied, as an additional assay, in case of doubt regarding the presence of deleted AMELY in the DNA profile.

2. Materials and methods

We have studied a total of 52 samples of oral swabs collected for forensic caseworks from phenotypically normal males and females, unrelated Caucasian individuals. The oral swabs were irreversibly anonymised (maintaining only the indication about the phenotypic sex), dried and then stored at room temperature.

The DNA was extracted using the Cst Forensic DNA kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

The sequences of primers used in the single PCR reaction are:
AMELf: 5′-ATCAGAGCTTAAACTGGGAAGCTG-3'
AMELr: 5′-CCCTGGGCTCTGTAAAGAATAGTG-3'
SRYf: 5′-TCCAGGAGGCACAGAAATTA-3'
SRYr: 5′-TCTTGAGTGTGTGGCTTTCG-3'

The PCR reaction mix was constituted by: 0.4 μM each of AMEL primers, 0.6 μM each of SRY primers, 1.25 U Taq polymerase (Applied Biosystems, Foster City, CA, USA), 0.2 μM each deoxyribonucleotidetriphosphates (dNTPs), 2.5 μl 10× Taq buffer (Applied Biosystems), 1 μl BSA (10 mg ml^{-1}) and 10 ng of template DNA in a final volume of 25 μl. To verify the efficiency of the amplification, a sample of human DNA has been used as positive control. Water instead of DNA was used as negative PCR control.

After an initial denaturation step of 1 min at 94 °C, the samples were amplified in an ABI 2720 Thermal Cycler, for 32 cycles of 20 s at 94 °C, 30 s at 59 °C and 20 s at 72 °C, followed by a final extension step of 7 min at 72 °C. The 15 μl amplified products were separated by electrophoresis at constant voltage (200 V) through a 12% polyacrylamide non-denaturing gel (12 × 10 cm) submerged in 10× Tris/borate/ethylene diamine tetraacetic acid (EDTA) (TBE) buffer, until xylene cyanol blue was at the end of the gel. The gel was stained in ethidium bromide solution (0.5 mg ml^{-1}) and visualised under ultraviolet (UV) light.

3. Results

In this study we have developed a PCR duplex for sex determination, amplifying the SRY and amelogenin fragments, easily pursued by direct visualisation on a small polyacrylamide gel of the bands corresponding to the two respective amplified fragments.

Under normal conditions, as expected, the female shows a band of 106 bp (AMELX) with no band corresponding to SRY (Fig. 1, lanes 1, 2 and 6); the male shows three bands respectively, 106 bp and 112 bp for AMELX and AMELY, and 197 bp for SRY (Fig. 1, lanes 3 and 5).

In the case of AMELY deletion, the sample shows two bands: 106 bp for AMELX and 197 bp for SRY (Fig. 1, lane 4). In the latter AMELY deletion implies that the male has an amplified band of 106 bp (AMELX), no band of 112 bp and one band of 197 bp corresponding to the SRY marker.

Our results showed that one individual among the 53 tested, although a phenotypically normal male, was genotyped as female using only the AMEL amplification, as demonstrated by the absence of the 112-bp band and presence of the 106-bp band. In order to determine if this anomaly was caused by AMELY deletion we assess the sex genotype adding amplification of another Y-specific marker.

The newly designed primers that amplified a 197-bp SRY fragment demonstrated that the individual carried the Y chromosome (Fig. 1, lane 4).

4. Discussion

Genotyping the X–Y homologous amelogenin gene segment for gender identification is widely used in biological material analysis in forensic casework.

Since both X- and Y-specific fragments can be amplified in a single reaction, amplification of the amelogenin gene offers the advantage, compared to specific Y markers, of having an internal positive control represented by the X-chromosome homologous fragment present in male and female samples.

However, the fallibility of the amelogenin-based sex test raises concern, as demonstrated by one of the first publications on the validity of this test.[37]

The consequences of erroneous gender determination may be noteworthy in different fields. The observed amelogenin sex-test failure rates in different populations should be considered, and an increasing contribution from population-studies in this field is desirable. It would be important to know the failure rate among different ethnic groups, including those which have not been studied to date.

We believe that analysis of the new SRY 197-bp fragment, associated with AMEL, could be very suitable for forensic purposes, because its small size avoids the possibility of preferential amplification in the duplex, and it may be useful in cases where doubtful results of amelogenin-based sex tests need confirmation of their validity.

Regarding the possible co-amplification for the SRY marker in concert with commercially available human STR kits,[31,32] we consider this a valid option; however it implies the adding of the SRY primer set in every amplification, while our method can be applied only when a confirmation is needed as an adjunct to standard gender typing.

The pitfalls in amelogenin-based sex determination may be overcome by adequate and common methodological approaches that involve co-amplification with other Y-chromosome markers.

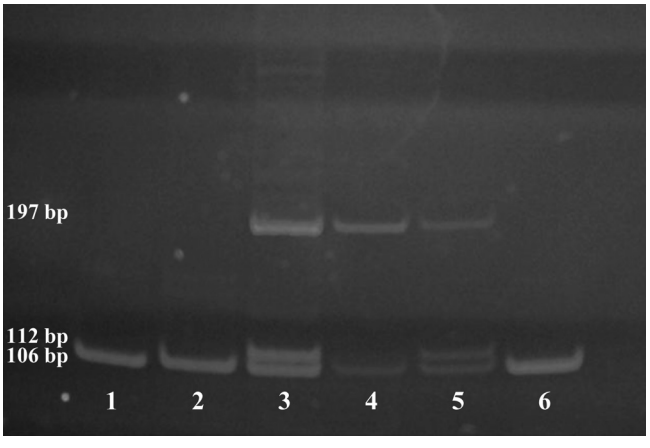

Fig. 1. AMEL (106/112 bp) + 97 bp SRY fragment from different buccal swabs samples. Lanes 1, 2 and 6 female; lanes 3 and 5 male; lane 4 AMELY deleted.

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Conflict of interest

None declared.

References

1. Nakahori Y, Takenaka O, Nakagome Y. A human X-Y homologous region encodes amelogenin. *Genomics* 1991;**9**:264–9.
2. Bayley DMD, Affra NA, Fergusons-Smith M. The X–Y homologous gene amelogenin maps to the short arms of both the X and the Y chromosomes and is highly conserved in primates. *Genomics* 1992;**14**:203–5.
3. Richard B, Delgado S, Gorry P, Sire JY. A study of polymorphism in human AMELX. *Arch Oral Biol* 2007;**52**:1026–31.
4. Sullivan KM, Mannucci A, Kimpton CP, Gill P. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *Biotechniques* 1993;**15**:636–41.
5. Mannucci A, Sullivan KM, Ivanov PL, Gill P. Forensic application of a rapid and quantitative DNA sex test by amplification of the X-Y homologous gene amelogenin. *Int J Legal Med* 1994;**106**:190–3.
6. von Wurmb-Schwark N, Bosinski H, Ritz-Timme S. What do the X and Y chromosomes tell us about sex and gender in forensic case analysis? *J Forensic Leg Med* 2007;**14**:27–30.
7. Santos FR, Pandya A, Tyler-Smith C. Reliability of DNA-based sex tests. *Nat Genet* 1998;**18**:103.
8. Hart PS, Vlaservich AC, Hart TC, Wright JT. Polymorphism (g2035C>T) in the amelogenin gene. *Hum Mutat* 2000;**15**:298.
9. Roffey PE, Eckhoff CI, Kuhl JL. A rare mutation in the amelogenin gene and its potential investigative ramifications. *J Forensic Sci* 2000;**45**:1016–9.
10. Henke J, Henke L, Chatthopadhyay P, Kayser M, Dülmer M, Cleef S, et al. Application of Y-chromosomal STR haplotypes to forensic genetics. *Croat Med J* 2001;**42**:292–7.
11. Thangaraj K, Reddy AG, Singh L. Is the amelogenin gene reliable for gender identification in forensic casework and prenatal diagnosis? *Int J Legal Med* 2002;**116**:121–3.
12. Steinlechner M, Berger B, Niederstätter H, Parson W. Rare failures in the amelogenin sex test. *Int J Legal Med* 2002;**116**:117–20.
13. Chang YM, Burgoyne LA, Both K. Higher failures of amelogenin sex test in an Indian population group. *J Forensic Sci* 2003;**48**:1309–13.
14. Chang YM, Perumal R, Keat PY, Yong RY, Kuehn DL, Burgoyne L. A distinct Y-STR haplotype for Amelogenin negative males characterized by a large Y(p) 11.2 (DYS458-MSY1-AMEL-Y) deletion. *Forensic Sci Int* 2007;**166**:115–20.

15. Kashyap VK, Sahoo S, Sitalaximi T, Trivedi R. Deletions in the Y-derived amelogenin gene fragment in the Indian population. *BMC Med Genet* 2006;**7**:37.
16. Michael A, Brauner P. Erroneous gender identification by the amelogenin sex test. *J Forensic Sci* 2004;**49**:258–9.
17. Lattanzi W, Di Giacomo MC, Lenato GM, Chimienti G, Voglino G, Resta N, et al. A large interstitial deletion encompassing the amelogenin gene on the short arm of the Y chromosome. *Hum Genet* 2005;**116**:395–401.
18. Mitchell RJ, Kreskas M, Baxter E, Buffalino L, Van Oorschot RA. An investigation of sequence deletions of amelogenin (AMELY), a Y-chromosome locus commonly used for gender determination. *Ann Hum Biol* 2006;**33**:227–40.
19. Cadenas AM, Regueiro M, Gayden T, Singh N, Zhivotovsky LA, Underhill PA, et al. Male amelogenin dropouts: phylogenetic context, origins and implications. *Forensic Sci Int* 2007;**166**:155–63.
20. Jobling MA, Lo IC, Turner DJ, Bowden GR, Lee AC, Xue Y, et al. Structural variation on the short arm of the human Y chromosome: recurrent multigene deletions encompassing amelogenin Y. *Hum Mol Genet* 2007;**16**:307–16.
21. Turrina S, Filippini G, Voglino G, De Leo D. Two additional reports of deletion on the short arm of the Y chromosome. *Forensic Sci Int Genet* 2011;**5**:242–6.
22. Kumagai R, Sasaki Y, Tokuta T, Biwasaka H, Aoki Y. DNA analysis of family members with deletion in Yp11.2 region containing amelogenin locus. *Leg Med (Tokyo)* 2008;**10**:39–42.
23. Kumagai R, Sasaki Y, Tokuta T, Biwasaka H, Matsusue A, Aoki Y, et al. Distinct breakpoints in two cases with deletion in the Yp11.2 region in Japanese population. *Hum Genet* 2010;**127**:537–43.
24. Takayama T, Takada N, Suzuki R, Nagaoka S, Watanabe Y, Kumagai R, et al. Determination of deleted regions from Yp11.2 of an amelogenin negative male. *Leg Med (Tokyo)* 2009;**11**(Suppl. 1):S578–80.
25. Shadrach B, Commane M, Hren C, Warshawsky I. A rare mutation in the primer binding region of the Amelogenin gene can interfere with gender identification. *J Mol Diagn* 2004;**6**:401–5.
26. Alves C, Coelho M, Rocha J, Amorim A. The amelogenin locus displays a high frequency of X homologue failures in Sao Tome Island (West Africa). In: Amorim A, Corte-Real F, Morling N, editors. *Progress in forensic genetics 11.* , Amsterdam: Elsevier Science; 2006. p. 271–3.
27. Caratti S, Voglino G, Cirigliano V, Ghidini A, Taulli R, Torre C, et al. Amplification failure of the amelogenin gene (AMELX) caused by a primer binding site mutation. *Prenat Diagn* 2009;**29**:1180–2.
28. Maciejewska A, Pawlowski R. A rare mutation in the primer binding region of the amelogenin X homologue gene. *Forensic Sci Int Genet* 2009;**3**:265–7.
29. Haas-Rochholz H, Weiler G. Additional primer sets for an amelogenin gene PCR-based DNA-sex test. *Int J Legal Med* 1997;**110**:312–5.
30. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003;**423**:825–37.
31. Drobnič K. A new primer set in a SRY gene for sex identification. In: Amorim A, Corte-Real F, Morling N, editors. *Progress in forensic genetics 11*. Amsterdam: Elsevier Science; 2006. p. 268–70.
32. Kastelic V, Budowle B, Drobnic K. Validation of SRY marker for forensic casework analysis. *J Forensic Sci* 2009;**54**:551–5.
33. Andréasson H, Allen M. Rapid quantification and sex determination of forensic evidence materials. *J Forensic Sci* 2003;**48**:1280–7.
34. Morikawa T, Yamamoto Y, Miyaishi S. A new method for sex determination based on detection of SRY, STS and amelogenin gene regions with simultaneous amplification of their homologous sequences by a multiplex PCR. *Acta Med Okayama* 2011;**65**:113–22.
35. Chen H, Lowther W, Avramopoulos D, Antonarakis SE. Homologous loci DXYS156X and DXYS156Y contain a polymorphic pentanucleotide repeat (TAAAA)n and map to human X and Y chromosomes. *Hum Mutat* 1994;**4**: 208–11.
36. Mukerjee S, Mukherjee M, Ghosh T, Kalpana D, Sharma AK. Differential pattern of genetic variability at the DXYS156 locus on homologous regions of X and Y chromosomes in Indian population and its forensic implications. *Int J Legal Med* 2013;**127**:1–6.
37. Brinkmann B. Is the amelogenin sex test valid? *Int J Legal Med* 2002;**116**:63.